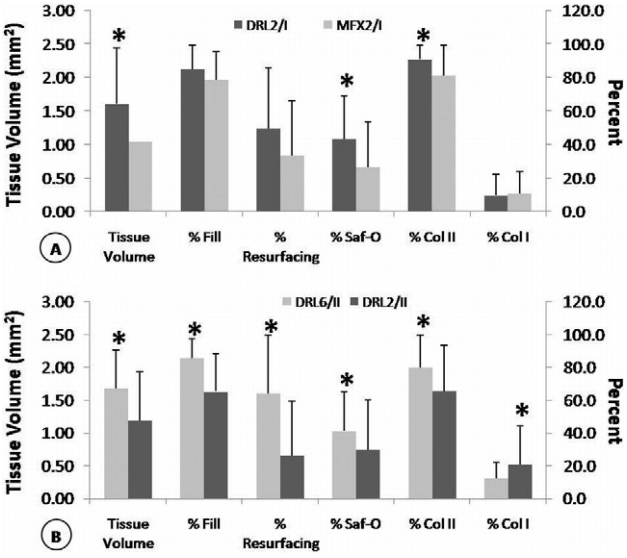

Figure 3. Effects of bone marrow stimulating procedure by microfracture (MFX2 holes vs drilling (DRL2 holes) to the same depth of 2 mm (A) and of the depth of drill holes (6 mm DRL6 vs 2 mm DRL2) (B) on tissue repair in cartilage defects from rabbit trochleas 90 days post-operatively. *$P<0.05$.

collagen type II and less collagen type I in the repair matrix ($P<0.04$ for all) (Fig. 3).

Conclusions: Surgical techniques affect the patterns and connectivity of subchondral bone marrow channels, thus influencing cartilage repair outcomes. Bone marrow stimulation by DRL provided free channels to marrow stroma and led to significantly better cartilage repair than MFX at 3 months. Compared to shallow perforation, deep DRL with increased access to marrow compartments produced more effective hyaline-like cartilage repair in rabbits. These findings suggest a surgical technique that cleanly removes bone and bone fragments and provides free access to marrow may be superior to MFX as a bone marrow stimulation technique for cartilage repair.

Moderated Poster Session 2

063

THE ROLE OF AKT1 IN TERMINAL STAGES OF ENDOCHONDRAL BONE FORMATION: ANGIOGENESIS AND OSSIFICATION

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Purpose: Longitudinal bone growth is the result of endochondral bone formation which takes place in the growth plate. The rate of chondrocyte proliferation and hypertrophy, vascular invasion with the formation of primary ossification centers and cartilage replacement by bone tissue are all important processes required for normal growth. We have shown a role for the PI3K signaling pathway in chondrocyte hypertrophy and bone growth in tibia explant cultures. In this current study we aimed to investigate the role of Akt1, an important target of PI3K, in endochondral ossification.

Methods: Mouse long bones were fixed in formaldehyde, paraffin embedded and sectioned. Different staining methods were applied: Safranin O/Fast green for cartilage visualization and TRAP stain for osteoclast activity. Immunohistochemistry was also performed using antibodies against vascular endothelial growth factor (VEGF) and matrix metalloproteinase 14 (MMP-14) in eleven day-old Akt1 KO and control long bones. Tibiae isolated from E15.5 mice were cultured for three weeks in the presence of a PI3K inhibitor (LY294002) or vehicle control. These bones were measures at the beginning and at the end of the time course. MicroCT analysis was performed in seven day- and one year-old Akt1 KO and control mice. Bone mineral density (BMD) and bone mineral content (BMC) were analyzed in the proximal tibia and in the 5th lumbar vertebrae of one year-old Akt1 mice.

Results: Akt1 KO mice showed reduced size compared to their littermates throughout life, but the largest difference in body size was observed around one week of age. Focusing on this specific developmental stage, we discovered delayed secondary ossification in the long bones of Akt1 KO mice. A delay in formation of a structure resembling a secondary ossification center (SOC) was also seen in tibia organ cultures treated with LY294002. The expression of MMP-14, the main protease responsible for development of secondary ossification centers, was decreased in the epiphysis of Akt1 KO mice, possibly explaining the delay in SOC seen in the Akt1 KO mice. BMD and BMC were found to be decreased in one year-old Akt1 KO mice, suggesting that the original delay in ossification affects bone quality in older animals.

Conclusions: We show a novel role for Akt1 protein kinase in the formation of long-bone SOC. The reduction in MMP-14 protein levels in the Akt1 KO mouse tissues suggested a regulatory mechanism possibly responsible for this delay in skeletal development.

064

DISTINCT TRANSCRIPTIONAL CONTROL OF CHONDROCYTE HYPERTROPHY AND CARTILAGE DEGRADATION BY C/EBP-BETA AND RUNX2 DURING endochondral ossification

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Purpose: Chondrocyte hypertrophy and cartilage degradation, characterized by expressions of type X collagen (COL10) and matrix metalloproteinase 13 (MMP13), respectively, are sequential and crucial steps in endochondral ossification during skeletal growth and osteoarthritis (OA) progression. This study investigated the role of CCAAT/enhancer-binding protein β (C/EBPβ) in chondrocytes and its interaction with Runx2 during the endochondral ossification.

Methods: To know the physiological functions of C/EBPβ and Runx2, we compared the skeletal phenotypes of the homozygous (–/–) or heterozygous (+/–) deficient mice with the respective wild-type littermates by Alcian blue, Alizarin red and von Kossa stainings, BrdU labeling, and immunostainings of COL10 and MMP13. After an experimental OA model was created surgically by inducing instability in the mouse knee joints, the articular cartilage underwent histological analyses as above and the cartilage destruction was quantified by the OARSI histopathology grading. For the functional analyses, we established stable lines of human chondrogenic SW1353 cells with retroviral transfection of C/EBPβ, Runx2, or both of them. Cell proliferation was assessed by CCK-8 assay. Chondrocyte differentiation was determined by Alcian blue and Alizarin red stainings, as well as expressions of COL10 and MMP-13 by real-time RT-PCR. Promoter activities of COL10 and MMP13 genes were analyzed by luciferase assays in SW1353 cells transfected with reporter constructs containing the respective promoter fragments, and the core responsive regions were determined by the deletion, mutagenesis, and tandem-repeat analyses of the constructs.

Results: C/EBPβ–/– mice exhibited dwarfism from embryonic stages with delayed chondrocyte hypertrophy and decreased

COL10 expression in the limb cartilage. Under the OA induction in the wild-type joints, C/EBPβ was induced at the frontline of cartilage destruction, whereas in the C/EBPβ+/– joints, the destruction as well as chondrocyte hypertrophy and COL10 expression were significantly suppressed. In the ex vivo culture of C/EBPβ–/– costal chondrocytes, COL10 expression was significantly decreased compared to the wild-type culture. The mRNA level and promoter activity of COL10 were enhanced by the C/EBPβ transfection, and the core responsive region of the COL10 promoter was identified between -81 and -76 bp relative to the transcriptional start site. Since C/EBPβ and Runx2 are known to function as mutual transcriptional co-factors, we further generated C/EBPβ and Runx2 compound deficient (C/EBPβ–/–; Runx2+/–) mice. The C/EBPβ–/–; Runx2+/– mice exhibited severer dwarfism than the C/EBPβ–/– mice. Although chondrocyte hypertrophy and COL10 expression were comparable between the two genotypes, cartilage degradation and MMP13 expression were markedly suppressed by the Runx2 insufficiency. In the culture of SW1353 cells, co-transfection of C/EBPβ and Runx2 enhanced MMP13 expression, but not proliferation or COL10 expression, as compared to a single transfection of C/EBPβ or Runx2. The promoter activity of MMP13 was synergistically enhanced by the co-transfection, and the core responsive region was identified between -111 and -89 bp, which contains a C/EBP-binding motif, but not a Runx2-binding motif.

Conclusions: C/EBPβ is a crucial transcription factor for chondrocyte hypertrophy and cartilage degradation. Runx2 contributes to the latter step as the co-factor, but not to the former step, indicating distinct transcriptional control of these sequential steps during endochondral ossification by C/EBPβ and Runx2.

065

MiR-140 IS EXPRESSED IN DIFFERENTIATED HUMAN ARTICULAR CHONDROCYTES AND MODULATES IL-1 RESPONSES

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Purpose: MicroRNAs (miRNAs) are a class of noncoding small RNAs that act as negative regulators of gene expression. The miRNAs exhibit tissue-specific expression patterns and changes in their expression may contribute to pathogenesis. The objectives of this study were to identify miRNAs expressed in articular chondrocytes, determine changes in osteoarthritic cartilage and address the function of miR-140.

Methods: To identify miRNAs specifically expressed in chondrocytes, we performed gene expression profiling using miRNA microarrays and quantitative PCR with human articular chondrocytes compared to human mesenchymal stem cells (MSC). The expression pattern of miR-140 was monitored during chondrogenic differentiation of hMSC in pellet cultures and in human articular cartilage from normal and osteoarthritic knee joints. We tested effects of IL-1β on miR-140 expression. Double-strand (ds) miR-140 was transfected into chondrocytes to analyze changes in the expression of genes associated with osteoarthritis.

Results: Microarray analysis showed that miR-140 has the largest difference in expression between chondrocytes and MSC. During chondrogenesis cultures of MSC miR-140 expression increased in parallel with Sox9 and Col2a1. Normal human articular cartilage expressed miR-140 and this was significantly reduced in OA tissue. In vitro treatment of chondrocytes with IL-1β suppressed miR-140 expression.In contrast to miR-140, miR-146 has a broader tissue distribution, it is increased in response to IL-1, it is upregulated in OA. Transfection of chondrocytes with ds-miR-140 downregulated IL-1β-induced ADAMTS-5 expression and rescued the IL-1β -dependent repression of Aggrecan gene expression.Moreover, we performed searches in three databases (“TargetScan”, “ PicTar”, “miRbase”) and this yielded 223-975 potential miR-140 targets. Only 9 potential targets were identified in all three databases.

Conclusions: This study shows that miR-140 has a chondrocyte differentiation-related expression pattern. The reduction in miR-140 expression in OA cartilage and in response to IL-1β may contribute to the abnormal gene expression pattern characteristic of OA.

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THE ADIPOKINE, RESISTIN, INDUCES A HIGH LEVEL OF EXPRESSION OF PRO-INFLAMMATORY CYTOKINES AND CHEMOKINES IN HUMAN ARTICULAR CHONDROCYTES

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Purpose: To provide a picture of the effect of resistin on human articular chondrocyte gene expression of cytokines and chemokines.

Methods: Chondrocytes were obtained from conserved area of cartilage from donors undergoing total knee joint replacement surgery. Chondrocytes were isolated and plated at a density of 2.5 ×105 cells/cm^2 in Dulbecco’s modified eagle’s medium (DMEM)/F12 media plus 10% fetal bovine serum (FBS), 50 mg/ml ascorbate and antibiotics (50 U/ml penicillin and 50 mg/ml streptomycin) for 24 h. Serum was removed and cells were allowed to recover for 24 h before adding resistin from BioVision (Mountain view, CA) for 24 h. Changes in gene expression were analyzed by quantitative real-time polymerase chain reaction.

Results: Resistin treated human articular chondrocytes showed significant increases in the expression of a large group of cytokines and chemokines, including IL-1a, IL-1b, IL-6, IL-8, CCL3, CL4, CCL8, CXCL1, CXCL3, CXCL6. As expected, the mRNA for matrix metalloproteinase (MMP)-1, MMP-3 and BMP2 also increased, but not as much as the above genes. Genes were placed in three